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Procedure in Field Testing Potato Seedlings for Leafroll Resistance

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Inoculating potato seedlings with leafroll-carrying green peach aphids.

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INTRODUCTION

Leafroll (*Corium solani* Holmes) has been responsible for serious losses in the yield of potatoes for a long period of time. Records referable to this virus disease are found from the earliest potato literature to the present. Losses due to leafroll vary considerably from season to season and from locality to locality, being greater on the whole toward the south or at lower elevations. Different varieties of potatoes vary considerably in their reaction to this disease. Several strains of the virus have been recognized recently (*12*). These strains vary as to severity of injury in any particular host. The disease has been an important cause of running out of potatoes and is, in large measure, responsible for various schemes for the production and certification of seed potatoes, especially in areas, mostly in the higher latitudes, where seed potatoes can be grown.

While the disease can be recognized from statements appearing in the early literature, it was not until 1914 that Orton (*5*) first described it. By 1920, work in Maine (*8*) and in Holland (*4*) demonstrated the transmission of the disease by aphids—especially the green peach aphid, *Myzus persicae* (Sulz.).

Subsequent work (e.g., *2, 6, 7, 9, 11*) has shown how the disease may be controlled to a great extent by roguing, early harvesting, and more recently through the control of the principal vector. All of these measures must be carefully employed every season to keep leafroll in check, and they are such that growers are usually unwilling to devote large acreages to the task. In some instances, technical help is available to growers as an aid in the production of foundation seed (*1*). The practical result is that adequate quantities of leafroll-free seed are almost never available to replace all runout stocks.

Genetic resistance to leafroll, if combined with other desirable qualities, offers a method of controlling the disease that should be simple,

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effective, and much less costly in both time and money than the present combination of practices.

Following an outbreak of leafroll in 1937 in Maine, an effort was made to discover natural resistance to this disease. This work showed that certain seedling varieties of potatoes possessed field resistance to leafroll (*3*). The indications were that the nature of the resistance was such that large numbers of seedlings would have to be tested in order to secure resistance and other desirable qualities in a new variety. Since field testing is somewhat uncertain, due to seasonal fluctuations in populations of the green peach aphid and to other factors not fully understood at present, it seemed desirable to develop a method of testing that would be more certain and that would, at the same time, permit the handling of large numbers of seedlings.

It is the purpose of this report to describe such a method and to discuss some of the general results obtained over a period of 8 years. A desirable leafroll resistant seedling has not yet been located, but sources of resistance have been found and a number of seedlings far more resistant than Katahdin, thus far the standard of comparison for field resistance to leafroll, have been selected.

SOURCE OF NEW SEEDLINGS

Field exposure to leafroll over a period of years indicated that certain differences in reaction to this virus could be observed, both in commercial varieties and in certain seedling populations. In particular, certain crosses between Imperia and Earlaine had been found to be even more resistant than Katahdin.

The new seedlings were developed as part of the National Potato Improvement Program and were obtained from F. J. Stevenson, Div. Fruit and Vegetable Crops and Diseases, Bur. Plant Industry, Soils and Agricultural Engineering, U. S. Dept. Agr. The crosses were made in the greenhouse at Beltsville, using parents that had demonstrated some resistance to leafroll. In some instances, both parents were from resistant lines while in others resistant sorts were selfed. These progenies, in family lines, were in turn tested and all susceptible seedlings were discarded. Through a series of recombinations, the factors for resistance have been concentrated to some extent and combinations with other desirable characters are now feasible. First generation tubers, in family lines, have been introduced at Aroostook Farm from Beltsville each spring.

GENERAL PROCEDURE IN TESTING NEW SEEDLINGS

The tubers to produce new seedlings have been planted, at the normal time, 2 feet apart in 34 inch rows to facilitate the harvesting of single hills. These tubers, while normally small in size, usually produce very satisfactory plants. Planting has been done in an isolated field to reduce field-to-field spread of virus diseases.

When the plants are from 4 inches to 8 inches high they are individually inoculated with viruliferous green peach aphids. These aphids are allowed to feed and reproduce on the growing plants for about one month after which they are destroyed with parathion. This is usually necessary to prevent direct damage to the plants from feeding injury due to large aphid populations. Such damage, if allowed to develop unchecked, interferes with readings of current season leafroll. These readings are the basis for eliminating susceptible plants in the first instance. All seedlings are sprayed, at intervals, with a fungicide to protect them from late-blight infection.

Shortly after the middle of August, in Maine, readings are made and all plants showing current season symptoms of leafroll are pulled up and the tubers then present are discarded. If plants are found showing symptoms of other virus diseases, as sometimes happens, such plants are also removed and any tubers found are discarded. Usually, of the current introductions, a rather high percentage (95 per cent or more) is discarded as susceptible to leafroll. The remaining hills are harvested during the last week in August and the tubers stored over winter.

In the second year of testing, a single tuber is selected from each hill saved and the plant (or plants) growing from this tuber is infested with viruliferous aphids. Five additional tubers are planted in another field as a means of checking on the accuracy of the virus reading made the previous August. In practice, a small percentage of plants deemed not to be showing current season symptoms of leafroll in August, are found, the following July, to be diseased.

This particular process is repeated for 5 seasons. Usually some additional hills are eliminated in the second or the third season, but there is less loss in the fourth and fifth years. Any hills surviving 5 inoculations are deemed to possess a high degree of resistance to leafroll.

At harvest time, in the field where the 5-hill lots are planted, promising seedlings are selected for increase and for further testing. These are increased in 10-hill lots, then in 20-hill lots, and, finally, if it is deemed worth while, they may be increased to the point where yield tests can be made. Meanwhile notes have been taken on other characteristics including vine type and reaction to late blight, scab, ring rot, and several mosaics.

Following satisfactory performance in yield tests, any surviving sorts are evaluated for cooking quality as French fries, potato chips, or boiled or baked potatoes, before being turned over to the trade for introduction following the standard procedure in Maine (*1*).

DETAILED TESTING

Method of inoculation. Leafroll can be transmitted only by grafting or by insects such as the green peach aphid. One practical method of inoculation for determining leafroll resistance is to expose the plants to the feeding of viruliferous aphids. Fortunately, the green peach aphid is easily propagated under greenhouse conditions, and there is no serious limitation to the numbers of aphids that can be produced, other than available greenhouse space.

A stock culture of the green peach aphid was established using migrants from wild Canadian plum, *Prunus nigra* Ait., the primary host of *Myzus persicae* (Sulz.) in this area. A number of secondary hosts are available for increasing populations in the greenhouse. Several of these have been tried but the most satisfactory one so far is the cultivated rutabaga. The variety "Alberger" is currently being used. Overwintered roots of rutabaga can be quickly forced to produce large plants.

Use of rutabagas for propagating aphids. Since fairly small rutabaga roots are most easily handled, it has been found desirable, in Maine, to delay outdoor seeding of the crop until about July 10. The resulting stands are thinned when the plants are from 4 inches to 6 inches tall so that the remaining plants are between 3 inches and 4 inches apart. The roots are harvested, in the fall, just before hard frosts are expected. By that time, because of the late planting date and close spacing, most roots are from 2 inches to 4 inches in diameter, although some are larger. These roots are topped high and stored in a cool place until the remains of the petioles of the cut-off leaves are ready to fall off. The roots are then cleaned and placed in shallow trays and held in cold storage over the winter. While there is some loss from storage rot, its effect is easily overcome by storing a quantity of roots somewhat in excess of anticipated needs. Rutabaga roots keep better if boron is added in the fertilizer, if this element is deficient in the soil.

Shortly before it is desired to increase aphid populations, the stored roots are placed in pots and allowed to grow in an aphid-free room in the greenhouse. Normally, the plants are held until the first flowers form before they are infested with aphids (Fig. 1). This practice provides a large amount of leaf area on which the aphids may multiply.

Size and care of flower pots for growing rutabagas. Greenhouse pots of various sizes have been used for growing rutabagas. Ten-inch

FIG. 1. Rutabaga plant at about the right stage for initial infestation with green peach aphids.

flower pots are quite satisfactory although both larger and smaller sizes have been used successfully. Since the pots need to be moved from the aphid-free room to another room where the aphids are produced, the weight of the pot and its soil is a matter of some importance. Pots smaller than 10 inches do not provide enough soil for the extensive root system of the rutabaga plants, while larger pots are unnecessarily heavy and take up somewhat more space on the bench.

It was found, when pots were watered from above, that there was considerable loss from rots. To overcome this, the use of braided glass-wool wicks ("Plant wick" #3 of the Raybestos Manhattan Company) was made standard practice. By placing the pots on 2 sticks over plant

FIG. 2. Potted rutabaga plant showing method of watering using #3 "Plant Wick."

pot saucers (Fig. 2), watering is automatic, and the top 2 or 3 inches of soil in a pot can be kept relatively dry at all times. The glass fiber wicks provide enough water to maintain very heavily infested plants. Figure 3 shows the method of placing the plant wick in the pot. In use, this would be covered with an additional 2 to 3 inches of soil.

Planting rutabagas. Since heavy aphid infestation can kill plants in a short time, it is necessary to have an adequate supply of potted rutabagas available at all times. This may be assured by potting rutabaga

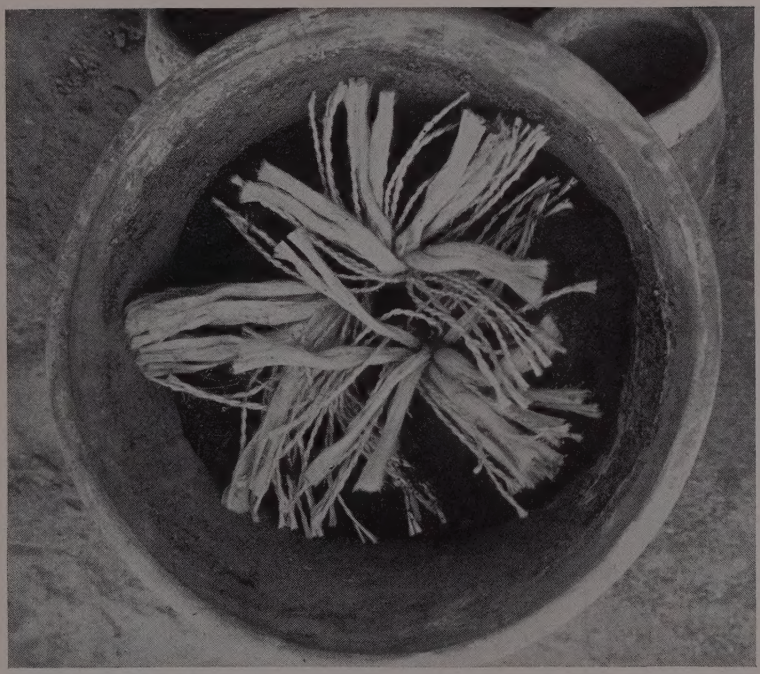

FIG. 3. Partially filled pot showing method of distributing "Plant Wick," before finally filling pot with soil, to insure adequate moisture up to the base of the rutabaga root which is placed just above the "Plant Wick."

roots at intervals so that, as soon as infested plants approach the point where aphids will soon kill them, their leaves may be stripped and placed on fresh plants. The aphids on the leaves of the older plants will then move to the new plants and continue to multiply. A single, well infested, plant can provide aphids for infesting from 15 to 20 additional plants. This factor must be taken into consideration in planning the planting schedule for the rutabaga plants. It is almost necessary to use rutabaga plants of the proper stage of growth at the time of initial infestation.

The number of transfers from rutabaga to rutabaga will be determined by the numbers of aphids needed for the field work. This, in turn, will depend on the number of potato seedlings to be tested and the speed with which the infesting of the potato plants can be carried out. In practice, a reserve supply should be maintained to offset losses due to adverse weather conditions which can prevent the prompt use of viruliferous aphids when they are ready for transfer to the field.

Care of aphids. Except for the period in the spring when rapid

multiplication of the aphids is in progress on rutabaga plants, the green peach aphid culture is maintained on cabbage. On this host, the green peach aphid reproduces slowly, especially during fall and winter, and the culture can be maintained throughout most of the year with relatively little attention.

Because hymenopterous parasites, especially *Aphidius nigriteleus* Smith, have caused trouble by attacking the green peach aphid in the greenhouse, it has been found desirable to protect the rutabaga plants with cloth covered cages (Fig. 4) during the time aphids are being increased. The cage offers protection against various predators as well.

Fungus diseases have attacked the aphid colonies during extended periods of humid weather, but this has usually happened toward the end of the active field season and has yet to have a seriously adverse influence on the work.

Procuring viruliferous aphids. Since green peach aphids reared on cabbage or rutabaga are not capable of transmitting leafroll, it is necessary to make a final transfer from rutabaga to potato plants known to be growing from tubers having the leafroll virus. Of the various potato varieties available, it has been found that leafroll Katahdin plants make the best growth for this particular purpose.

The leafroll Katahdin plants are allowed to grow to the stage where flower buds are just forming before they are infested by the transfer of aphids from rutabaga leaves. The potato plants may be grown to advantage in 7 inch flower pots—one seed piece to each pot. Three such pots fit under each of the cloth cages. Cages are used here also to protect the aphid colonies from parasites and predators. The potato plants are also planted in succession so that plants in the right stage of growth will be available for infesting at the proper time.

The potato plants are infested 10 days before they are to be taken to the field. This permits the development of another generation of the aphids and so materially increases the numbers of aphids available for the field work. It also insures an adequate interval on the leafroll plants so that all aphids acquire the ability to transmit leafroll.

The potato plants are rather heavily infested from rutabaga. However, care must be taken not to infest too heavily because Katahdin is easily injured by the feeding of the green peach aphid and its leaves will drop if too many aphids develop during the 10-day interval.

Inoculating potatoes in field. In practice, it has been found that under ideal conditions a group of 3 workers can distribute aphids from the potato plants growing in 21 pots to about 1500 seedlings in the course of one day. Time is also available for making the necessary transfers in the greenhouse to assure a supply of aphids 10 days hence.

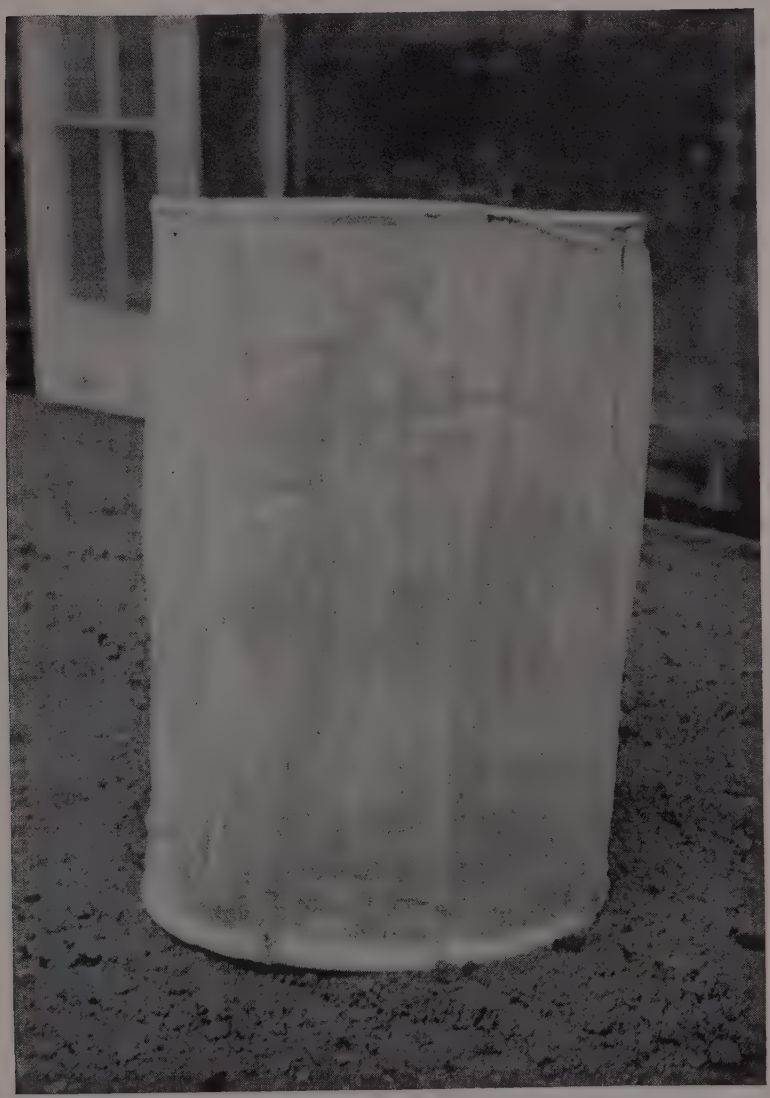

FIG. 4. Type of cloth covered cage used for the protection of aphid cultures on rutabaga and on leafroll potato in the greenhouse.

It is essential that the infested potato plants be supplied with adequate moisture at all times during the 10-day interval between initial

infestation and use. Plunging the pots into a greenhouse bench is of help in this connection.

While there is some variation in the growth of seedlings produced by first generation tubers due to variation in size of tubers, and to other factors, most plants grow rapidly and are large enough to be infested with viruliferous aphids toward the end of June in Maine. If leafroll Katahdin plants are first infested with aphids a few days after the potatoes to be tested have broken ground, most of these plants will be between 4 inches and 6 inches high 10 days later and field infestation can be started. The plants to be retested, because of the larger tubers planted, will normally be farthest along and work can be begun with them, in order to give the new introductions a little longer to grow before they are infested. In practice, plants too small to infest have been staked and infested later, after the field has been traversed the first time. Usually, a small percentage of late germinating plants must be infested still later in the season.

The potted Katahdin plants (leafroll source) are taken to the field where a group of 3 workers transfers aphids to each plant to be tested for resistance. One individual keeps the other 2 supplied with material. One of the other 2, equipped with a pair of curved surgical scissors, cuts off bits of leaf tissue on which are a minimum of 5 aphids. Previous tests (20) had shown that 5 aphids will assure nearly 100 per cent transmission of leafroll in such varieties as Green Mountain. The aphids are effective vectors of leafroll before they reach maturity so it is not necessary to distinguish between late instar nymphs and adults in determining that at least 5 individuals are present on each bit of leaf tissue. The actual amount of leaf area cut off will, of course, depend on the degree of infestation. It is rarely necessary to use more than a whole leaf and usually each leaflet may be cut into a number of pieces.

The third member of the team takes the aphid-bearing leaf tissue and places it on the plant to be tested. This is done rather rapidly but care must be taken not to injure the aphids and to make sure that the leaf tissue is placed in such a way that it will remain on the test plant until it has dried enough to force the aphids to seek feeding locations on the test plant. The most satisfactory location for placing the aphid-bearing tissue is found near the growing tip where the bit of leaf tissue may be inserted near the base of one of the partly expanded leaves. Since most such leaves have not yet assumed a horizontal position, the angle formed with the main stem is acute and usually the leaf must be pressed aside slightly to permit the insertion of the aphid-bearing leaf tissue. There is usually enough pressure to hold the bit of tissue in place temporarily, especially if it is cupped at the time of insertion. Even fairly brisk winds

dislodge very few bits of leaf tissue if they have been carefully placed. If the relative humidity is low and the day is bright, the tissue dries in a few hours and the aphids transfer to the test plant quickly. Under less favorable conditions, a longer time may be required.

It is obvious that this method of inoculation is more severe than is likely to occur under natural conditions at that time of year when aphids are not numerous. It is also much more certain since every plant is infested with aphids. By retesting for a period of 5 years, chance escapes are practically eliminated.

Readings for leafroll. Susceptible plants inoculated with leafroll when from 4 inches to 6 inches high will show current season symptoms of the disease in a short space of time—if the plants are growing rapidly sometimes in less than 2 weeks from the date of inoculation. In practice, in excess of 90 per cent of new introductions may be eliminated the first year. Readings in Maine can be made at any convenient interval after the latter part of July. It has been the practice to pull up plants showing current season leafroll, leaving for harvesting and storing only those plants not showing definite symptoms. A final check is made during the third week in August just prior to harvesting which is normally completed before September first. Tubers from individual hills are stored during the winter in labelled cloth bags.

Record keeping. These procedures have been kept as simple as possible because of the large number of potato varieties involved. In the first year, no attempt is made to distinguish individual hills until harvest time. Then, numbers are assigned to each remaining hill in each cross. This number, which consists of 2 parts—the cross number and the hill number—is used until the variety is later eliminated or named.

In the field, in order to simplify record keeping, stake numbers are assigned to each variety and these are used during the growing season. Any hills remaining after the end of 2 years, or more, of testing are carried over the winter under the combination number assigned at the end of the first year. In practice, one stake is used for each 5 hills. New introductions are planted in blocks 6 rows wide and as long as necessary. This facilitates harvesting operations at the end of the season.

SUMMARY OF RESULTS

Resistance to the field spread of leafroll is a desirable character which can be incorporated into commercial potato varieties through a breeding program. A method of testing for resistance on a large field scale is presented. It consists essentially of inoculating seedlings with viruliferous green peach aphids and, by this means, sorting out resistant seedlings which may then be screened for other desirable characteristics.

In the first few years, the progenies tested were relatively unselected and a high percentage of all plants was eliminated in the first year of testing. As survivors from the field test at the Experiment Station farm at Highmoor Farm and from the aphid test at Aroostook Farm were used more as parents, the percentage of survival became greater, indicating that some resistance was being secured.

More recently, the percentage of survival has trended downward again as an attempt has been made to make crosses that will have commercial acceptability. This is believed to indicate that inheritance of resistance is somewhat uncertain and that desirable characteristics, including resistance, will not be easily obtained. This, however, does not invalidate the method of testing.

The results to date from the testing of more than 51,000 seedlings have not yet provided a desirable commercially acceptable variety but they have indicated where resistance to leafroll may be found. Recombinations of characters, including resistance to leafroll, should eventually result in a variety that will greatly reduce losses due to the excessive spread of the leafroll virus.

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